#17 M.M. 10/8/02

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Borje S. Andersson

Serial No.: 09/415,890

Filed: October 8, 1999

For: PARENTERAL PIMARICIN AS TREATMENT OF SYSTEMIC INFECTIONS

Group Art Unit: 1616

Examiner: Neil Levy

Atty. Dkt. No.:UTXC:528–1/DLP

DECLARATION OF BORJE S. ANDERSSON UNDER 37 C.F.R. §1.132

I, DR. BORJE S. ANDERSSON, DECLARE AS FOLLOWS:

1. I am the inventor of the subject matter of the captioned patent application Serial No. 09/415,890.

2. It is my understanding that the Examiner in charge of the captioned application has rejected claims and required an identification of how much organic solvent is removed from the solvent vehicles of the invention.

3. Gas-chromatography/mass-spectroscopy (GC/MS) analysis of the instant solvent vehicles was performed under my supervision to determine how much organic solvent is remaining in the vehicles. A summary of this data is presented below and the actual data obtained in these studies are attached herewith as **Exhibit B**.

4. Gas chromatographic studies, such as the GC/MS methods described herein are typically used in the art as assays to detect the presence of residual organic solvents in compositions such as the instant solvent vehicles. These assays are extremely sensitive and are known to detect organic solvents as low as 10-100 ng/mL. For examples, see (Mulligan *et al.*, 1995, *J. Chromatogr. Sci.* 33:49-54, abstract submitted as **Exhibit C**; Camarasu *et al.*, 1998, *Pharm. Biomed. Anal.* 18:623-638, abstract submitted as **Exhibit D**; Li *et al.*, 2002, submitted as **Exhibit E**; that describe the use of gas-chromatography-mass spectroscopy as standard methods to detect the presence of residual solvents.

5. GC/MS was performed on samples of the instant solvent vehicles, exemplified by N'N-dimethlyacetamide (DMA) as a dipolar aprotic solvent and Intralipid™ (an aqueous lipid emulsion) as a secondary aqueous solvent. In some cases a drug with low aqueous solubility, exemplified by pimaricin, was also added to the solvent vehicle. The standard curve for comparison was prepared using DMA and hexane. Other characteristics of the GC/MS are as outlined below:

GC Injector: 200°C
Oven temperature: 40°C, 2min; at 5°C /min, to 70°C.
Column: ZB-1 15m x 0.25mm x 0.25µm capillary column
GC-MS transfer line: 290°C
Ion source temperature: 250°C
GC-MS Scan range: 30-500 Dalton

6. The data obtained are set forth in Table 1.

Table 1. GC/MS Analysis of the Solvent Vehicle

Sample Number	Sample Name	DMA (t_R) min	Peak Area	Inj. Vol. µl	GC-MS filename	DMA, %
1	1A	2.27	18576262	1.0	DMA 1A 091302_17	
2	1B	2.27	18087068	1.0	DMA 1B 091302_18	
3	2A	2.27	16525558	1.0	DMA 2A 091302_19	
4	2B	2.28	18284694	1.0	DMA 2B 091302_20	
5	3A	2.27	16866754	1.0	DMA 3A 091302_21	
6	3B	2.27	16422300	1.0	DMA 3B 091302_22	
7	I[$]	-	0	1.0	DMA I 091302_30	0%
8	II[$]	2.27	298308	1.0	DMA II 091302_31	1.70%
9	III[$]	2.29	199250	1.0	DMA III 091302_32	1.10%
10	IV with drug[#]	2.29	183498	1.0	DMA IV 091302_33	1.00%
11	V with drug[#]	-	0	1.0	DMA V 091302_34	0.00%
12	VI with drug[#]	-	0	1.0	DMA VI 091302_35	0.00%
13	std crv 1.0%*	2.46	184676787	1.0	DMA std 1% 091302_24	
14	std crv 2.5%*	2.68	457137954	1.0	DMA std 2.5% 091302_25	
15	std crv 5.0%*	2.91	920448678	1.0	DMA std 5% 091302_26	
16	std crv 50%*	2.69	12733869573	1.0	DMA std 50% 091302_28	

[$] The sample numbered 7-9 refer to the "solvent vehicle".
[#] In sample numbers 10-12 "drug" refers to the addition of pimaricin as an example of a drug with low aqueous solubility that was solubilized using the present solvent vehicle.
* The samples for the standard curve (std crv), numbered 13-16, were prepared bymixing DMA and Hexane (1:1) (Li *et al.*, 2002).

7. The samples numbered 1, 2, 3, 4, 5, and 6 in Table 1 were prepared by mixing DMA and Intralipid™ (1ml and 5 ml), 2 ml of which was analyzed for DMA content by GC/MS prior to lyophilization. These samples are designated as having 100% DMA content.

8. The samples numbered 7, 8 and 9 were prepared as described above for samples 1-6 by mixing DMA and Intralipid™ (1ml and 5 ml), 2 ml of which was then lyophilized for 36 hours, followed by reconstitution in 2ml of saline prior to GC/MS analysis.

9. The samples numbered 10, 11 and 12 were prepared by mixing the dipolar aprotic DMA (1ml) with the drug pimaricin at a concentration of 10 mg/ml. This was followed by mixing Intralipid™ (5 ml). 2 ml of this was then lyophilized for 36 hours, followed by reconstitution in 2ml of saline prior to GC/MS analysis.

10. The samples numbered as 13, 14, 15 and 16 in Table 1 were used to plot a standard curve and demonstrate the linearity of the GC/MS technique for detection of DMA. Samples 13-16 were prepared by mixing 200µl of DMA and 200µl of hexane by vortexing for 2 minutes. The mixture was centrifuged for 5 minutes at 2500 rpm, 100µl of the organic phase was obtained and 1.0 µl of this was injected into the GC/MS. Samples 13-15 were further diluted with hexane to obtain different DMA concentrations of 1.0, 2.5, and 5.0% respectively. These samples were then subject to GC/MS to obtain a standard curve that indicates the linearity for detection of DMA by GC/MS. This is also graphically displayed in Exhibit B.

11. As can be seen in Table 1:

A. Samples that were not lyophilized, represented by sample numbers 1-6, have a peak area of about 16422300 to 18576262 (see column 4 of Table 1) which is designated as having 100% of DMA.

B. Samples that were subject to lyophilization, represented by sample numbers 7-12, have a peak area of about 0 in three cases out of six cases and peak areas of 298308, 199250 and 183498 in three other cases. This represents 0% of DMA content for sample numbers 7, 11 and 12 and 1.7% DMA for sample number 8, 1.1% DMA for sample number 9, and 1.0% DMA for sample number 10 respectively (see data in column 7, Table 1).

12. Thus, 3 out of 6 samples had no detectable DMA, indicating that all the DMA was eliminated from these samples while the remaining three samples had 1.7%, 1.1% and 1.0% of DMA indicating that only trace amounts of DMA is retained or that virtually all the DMA is eliminated from these samples. Thus, 98% or more of the organic solvent can be removed from the solvent vehicles by the methods described in the above-referenced patent application.

13. All statements made in this Declaration of my own knowledge are true and all statements made in this Declaration on information and belief are believed to be true, and these statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both under 18 U.S.C. §1001 and may jeopardize the validity of this application or any patent issuing thereon.

September 18, 2002

Date

Borje S. Andersson